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7   **ACCURACY AND RELIABILITY ANALYSIS:**

8   • **Isolated Rabbit Eye (IRE) Test Method**

9   • **Isolated Chicken Eye (ICE) Test Method**

10   • **Bovine Corneal Opacity and Permeability (BCOP) Test Method**

11   • **Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test**

12   **Method**

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23   **SECTION I**

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25   **ISOLATED RABBIT EYE (IRE) TEST METHOD**

26   **ACCURACY AND RELIABILITY REANALYSIS**

27   **SUMMARY**

28

29   **1.0   INTRODUCTION**
30
31   **Table I-1.   Summary of IRE Database Changes**
32

Data Source	Data Set	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
			EPA[1]	EU[2]	GHS[3]	
			Cat[4] I/Total[5]	R41/Total	Cat 1/Total	
CEC (1991)[6]	New[7]	21	-	5/15	-	Six substances were excluded from the original database (n=21) because their EU classification was based on pH extreme or skin corrosivity information rather than *in vivo* rabbit eye test data.
	Old[7]	21	-	11/21	-	
Balls et al. (1995)	New	59	19/53	19/49	22/54	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix I-A**).
	Old	59	20/54	21/59	22/56	
Gettings et al. (1996)	New	25	17/25	16/24	16/24	The increase in the number of corrosive/severe irritants is due to the reclassification of several substances based on the presence of ocular damage at day 21 post-treatment.
	Old	25	12/25	12/25	12/25	
Guerriero et al. (2004)	New	44	11/38	11/38	11/38	Six substances were excluded from the original database because their classification was based on pH extremes or skin corrosivity information rather than *in vivo* rabbit eye test data.
	Old	44	16/41	15/41	16/41	
Expanded Data Set[8]	New	91[1]	31/76	37/80	33/76	From 11-15 substances were excluded from the original database, because specific regulatory classification criteria were not met (e.g., persistence could not be determined due to study termination).

33   [1]EPA = U.S. Environmental Protection Agency (EPA [1996]).
34   [2]EU = European Union (EU [2001]).
35   [3]GHS = Globally Harmonized System (UN [2003]).
36   [4]Cat = Category.
37   [5]Number of severe irritants by regulatory classification/number of classifiable substances.

38   [6]When the same substance was evaluated in multiple laboratories, the IRE ocular irritancy potential for each independent test result was determined.
39   Subsequently, an overall IRE ocular irritancy classification was assigned for each substance based on the majority of ocular irritancy classification calls and this
40   call was used in the analysis of IRE test method accuracy (approach described in **Section I-2.1**).
41   [7]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in the draft IRE BRD.
42   [8]Includes the 38 substances tested by Guerriero et al. (2004) that could be classified and additional substances classified as severe irritants from CEC (1991) (EU
43   classification system only), Balls et al. (1995), and Gettings et al. (1996), based either on an *in vitro* corneal opacity score of at least 3.0 or an *in vitro* corneal
44   swelling of at least 25%; these were among the criteria used by Guerriero et al. (2004) to identify corrosive/severe irritants.

45 **2.0   ACCURACY OF THE IRE TEST METHOD - REANALYSIS**
46
47 **Table I-2.   Evaluation of the Performance of the IRE Test Method In Predicting Ocular Corrosives and Severe Irritants**
48 **Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS[1] Classification System, by Study and**
49 **Overall**
50

Data Source	Data Set	N[2]	Accuracy		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
			%	No.[3]	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Balls et al. (1995)[4,5]	New[6]	54/59	54	29/54	68	15/22	44	14/32	45	15/23	67	14/21	56	18/32	32	7/22
	Old[6]	56/59	50	28/56	64	14/22	41	14/34	41	14/34	64	14/22	59	20/34	36	8/22
Gettings et al. (1996)	New	24/25	67	16/24	63	10/16	75	6/8	83	10/12	50	6/12	25	2/8	38	6/16
	Old	25/25	64	16/25	56	9/16	78	7/9	82	9/11	50	7/14	22	2/9	44	7/16
Guerriero et al. (2004)	New	38/44	79	30/38	100	11/11	70	19/27	58	11/19	100	19/19	30	8/27	0	0/11
	Old	36/44	78	28/36	100	12/12	67	16/24	60	12/20	100	16/16	33	8/24	0	0/12
Expanded Data Set[7]	New	76/91	68	52/76	100	33/33	44	19/43	58	33/57	100	19/19	56	24/43	0	0/33

51 [1]GHS = United Nations Globally Harmonized System (UN [2003]).
52 [2]N = number of substances included in this analysis/the total number of substances in the study.
53 [3]Data used to calculate the percentage.
54 [4]One chemical (benzalkonium chloride, 1%) was tested *in vivo* twice within the same laboratory. The results were discordant with respect to GHS classification;
55 the analysis was performed assuming Category 1 classification.
56 [5]Performance calculated using the overall *in vitro* classification based on the majority and/or most severe classification among the four laboratories.
57 [6]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on analysis included in the draft IRE BRD with corrections.
58 [7]Includes the 38 substances tested by Guerriero et al. (2004) that could be classified and 38 additional substances classified as severe irritants from Balls et al.
59 (1995) and Gettings et al. (1996), based either on an *in vitro* corneal opacity score of at least 3.0 or an *in vitro* corneal swelling of at least 25%; these were among
60 the criteria used by Guerriero et al. (2004) to identify corrosive/severe irritants. When the same substance was evaluated in multiple laboratories, the IRE ocular
61 irritancy potential for each independent test result was determined. Subsequently, an overall IRE ocular irritancy classification was assigned for each substance
62 based on the majority of ocular irritancy classification calls and this call was used in the analysis of IRE test method accuracy (approach described in **Section I-**
63 **2.0**); this process reduced the total number of substances in the expanded data set to 76 for the GHS classification system (UN [2003]).

64 **Table I-5.** **False Negative and False Positive Rates of the IRE Test Method, by**
65 **Chemical Class and Properties of Interest, for the GHS[1] Classification**
66 **System (Analysis Based on the Expanded Data Set)**
67

Category	N^2	False Positive Rate[3]		False Negative Rate[4]	
		%	No.[5]	%	No.
Overall	76	56	24/43	0	0/33
Chemical Class[6]					
Alcohol	11	60	6/10	0	0/1
Amide	5	0	0/3	0	0/2
Amine	9	60	3/5	0	0/4
Carboxylic acid	5	67	2/3	0	0/2
Ester	6	67	4/6	-	0/0
Ether	8	40	2/5	0	0/3
Formulation	12	100	2/2	0	0/10
Heterocycle	16	50	4/8	0	0/8
Ketone	6	67	4/6	-	0/0
Onium compound	9	33	1/3	0	0/6
Sulfur compound	7	20	1/5	0	0/2
Properties of Interest					
Liquid/Solution	43	83	19/23	0	0/20
Solid	33	25	5/20	0	0/13
Surfactant – Total	10	50	2/4	0	0/6
-nonionic	3	50	1/2	0	0/1
-anionic	-	-	-	-	-
-cationic	7	100	1/1	0	0/6
pH – Total[7]	0	-	-	-	-
- acidic (pH < 7.0)	0	-	-	-	-
- basic (pH > 7.0)	0	-	-	-	-
NICEATM GHS Category 1 Subgroup[8]	21	-	-	0	0/0
- Total	4	-	-	0	0/4
- 4 (CO=4 at any time)	3	-	-	0	0/3
- 3 (severity/persistence)	2	-	-	0	0/2
- 2 (severity)	*9*	-	-	0	0/9
- 2-4 combined[9]	12	-	-	0	0/12
- 1 (persistence)					

68 [1]GHS = United Nations Globally Harmonized System (UN [2003]).
69 [2]N = number of substances.
70 [3]False Positive Rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.
71 [4]False Negative Rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.
72 [5]Data used to calculate the percentage.
73 [6]Chemical classes included in this table are represented by at least five substances tested in the IRE test method
74 and assignments are based on the MeSH categories (www.nlm.nih.gov/mesh). See **Appendix B**.
75 [7]Total number of GHS Category 1 substances for which pH information was available.
76 [8]Subgroups assigned based on the whether classification as a GHS Category 1 substance was based on severity
77 and/or persistence. 1: based on lesions that are persistent; 2: based on lesions that are severe (not including
78 Corneal Opacity [CO]=4); 3: based on lesions that are both severe (not including CO=4) and persistent; 4: CO =
79 4 at any time.
80 [9]Subcategories 2 to 4 combined to allow for a direct comparison of GHS Category 1 substances classified *in*
81 *vivo* based on some lesion severity component and those classified based on persistent lesions alone.
82
83

83   **3.0   RELIABILITY OF THE IRE TEST METHOD - REANALYSIS**
84
85   **3.2   Reanalysis of IRE Test Method Intralaboratory Repeatability**
86
87   No additional data were received that would enable an analysis of intralaboratory

88   repeatability.
89
90   **3.3   Reanalysis of IRE Test Method Intralaboratory Reproducibility**
91
92   No additional IRE data has been received that would enable an evaluation of intralaboratory

93   reproducibility.
94
95   **3.4   Reanalysis of IRE Test Method Interlaboratory Reproducibility**
96
97   **Table I-7.   Interlaboratory Variability of Balls et al. (1995) for Substances Classified**
98   **   as Ocular Corrosives/Severe Irritants or Nonsevere Irritants/Nonirritants**
99   **   Using the GHS[1] Classification System**
100

Classification (*in vivo*/ *in vitro*)[2]	Data Set	Number of Substances	Number of Testing Labs	Substances with 100% Agreement Among Labs	Substances with 75% Agreement Among Labs	Substances with 50% Agreement Among Labs
+/+	New[3]	14	4	14 (100%)	0 (0%)	0 (0%)
	Old[3]	14	4	14 (100%)	0 (0%)	0 (0%)
+/-	New	9	4	5 (55%)	4 (44%)	0 (0%)
	Old	8	4	4 (50%)	4 (50%)	0 (0%)
-/+	New	20	4	8 (40%)	3 (15%)	9 (45%)
	Old	20	4	8 (40%)	3 (15%)	9 (45%)
-/-	New	14	4	6 (43%)	8 (57%)	0 (0%)
	Old	14	4	6 (43%)	8 (57%)	0 (0%)
?/-	New	1	4	1 (100%)	0 (0%)	0 (0%)
	Old	2	4	2 (100%)	0 (0%)	0 (0%)
?/+	New	1	4	1 (100%)	0 (0%)	0 (0%)
	Old	1	4	1 (100%)	0 (0%)	0 (0%)
TOTAL	New	59	4	35 (59%)	15 (25%)	9 (15%)
	Old	59	4	35 (59%)	15 (25%)	9 (15%)

101   [1]GHS = Globally Harmonized System (UN [2003]).
102   [2]A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant
103   (Category 1); a "-" indicates that the substance was assigned an overall classification of nonsevere irritant
104   (Category 2A, 2B) or nonirritant; a "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies
105   were terminated too early to assess reversibility of effects), a GHS classification could not be made. See
106   **Section 2.0** for a description of the rules followed to classify the ocular irritancy of test substances tested
107   multiple times *in vitro*.
108   [3]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous
109   analysis included in the draft IRE BRD.
110

111   The quantitative analysis of interlaboratory reproducibility was not affected by the

112   information received subsequent to the release of the draft IRE BRD, and therefore is not

113   presented here (see draft IRE BRD, November 1, 2004).

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116   **3.5   IRE Test Method Historical Positive and Negative Control Data - Reanalysis**
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118   As detailed in the draft IRE BRD, historical control data has not been provided for this

119   evaluation (November 1, 2004).

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130 **SECTION II**

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132 **ISOLATED CHICKEN EYE (ICE) TEST METHOD**

133 **ACCURACY AND RELIABILITY REANALYSIS**

134 **SUMMARY**

135

136   **1.0   INTRODUCTION**
137
138   **Table II-1.   Summary of ICE Database Changes**
139

Data Source	Data Set	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
			EPA[1]	EU[2]	GHS[3]	
			Cat[4] I/Total[5]	R41/Total[4]	Cat 1/Total[4]	
Prinsen and Koëter (1993)	New[6]	21	2/10	7/21	2/10	The decrease in the number of corrosive/severe irritants is due to the reclassification of one substance from a severe ocular irritant/corrosive to a moderate ocular irritatnt.
	Old[6]	21	3/10	8/21	3/10	
Balls et al. (1995)	New	59	19/51	19/50	22/54	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A**).
	Old	59	20/54	21/59	22/56	
Prinsen (1996)	New	44	2/36	2/36	2/36	The *in vivo* data that corresponded to the substances tested were received, which allowed for an evaluation of all three regulatory hazard classification systems for this study (previously, the analysis of severe irritants was limited to the published EU classification for these substances). The published EU classification for four severe irritants was based only on dermal corrosivity (no rabbit eye test was performed). Therefore, these substances were excluded from the revised analysis.
	Old	44	0/29	6/44	0/29	
Prinsen (2000)	New	4	-	1/4	-	This is new information received subsequent to the original analysis. Because the corresponding *in vivo* rabbit test data were not submitted, the analysis was based on the provided EU classification only.
Prinsen (2005)	New	50	4/46	4/46	4/46	This is new information received subsequent to the original analysis. Four of these substances were classified based only on dermal corrosivity (no *in vivo* rabbit eye test was performed); these substances were excluded from the analysis.

140   [1]EPA = U.S. Environmental Protection Agency (EPA [1996]).
141   [2]EU = European Union (EU [2001]).
142   [3]GHS = Globally Harmonized System (UN [2003]).
143   [4]Cat = Category.
144   [5]First number (before forward slash) refers to the number of substances in each study that were classified as a severe irritant according to each classification system (EPA, EU,
145   and GHS). The second number (after the forward slash) refers to the number of substances that were classified, based on animal data, for each classification system (EPA, EU,
146   GHS).
147   [6]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in the draft ICE BRD.

148 **2.0   ACCURACY OF THE ICE TEST METHOD - REANALYSIS**
149
150 **Table II-2.   Evaluation of the Performance of the ICE Test Method In Predicting Ocular Corrosives and Severe Irritants**
151 **Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS[1] Classification System, by Study and**
152 **Overall**

Data Source	N[2]	Accuracy		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
		%	No.[3]	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Prinsen and Koëter (1993) (new)[4]	10/21	80	8/10	100	2/2	75	6/8	50/2/4	3/4	100	6/6	25	2/8	0	0/2
Prinsen and Koëter (1993) (old)[4]	10/21	80	8/10	100	3/3	86	6/7	75	3/4	100	6/6	17	1/7	0	0/3
Balls et al. (1995)[5,6] (new)	54/59	69	37/54	50	11/22	81	26/32	65	11/17	70	26/37	19	6/32	50	11/22
Balls et al. (1995)[5,6] (old)	56/59	71	40/56	55	12/22	82	28/34	67	12/18	74	28/38	18	6/34	46	10/22
Prinsen (1996) (new)	36/44	97	35/36	50	1/2	100	34/34	100	1/1	97	34/35	0	0/34	50	1/2
Prinsen (1996) (old)	29/44	100	29/29	-	0/0	100	29/29	-	0/0	100	29/29	0	0/29	-	0/0
Prinsen (2005) (new)	46/50	89	41/46	0	0/4	98	41/42	0	0/1	91	41/45	2	1/42	100	4/4
Entire Data Set[6,7] (new)	144/171	83	120/144	50	15/30	92	105/114	63	15/24	88	105/120	8	9/114	50	15/30
Entire Data Set[6,7] (old)	92/121	82	75/92	60	15/25	90	60/67	68	15/22	86	60/70	10	7/67	40	10/25

153 [1]GHS = Globally Harmonized System (UN [2003]).
154 [2]N = Number of substances included in this analysis/the total number of substances in the study.
155 [3]No. = Data used to calculate the percentage.
156 [4]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in the draft ICE BRD.
157 [5]One chemical (benzalkonium chloride, 1%) was tested *in vivo* twice within the same laboratory. The results were discordant with respect to GHS classification; the analysis was
158 performed assuming Category 1 classification.
159 [6]Performance calculated using the overall *in vitro* classification based on the majority and/or most severe classification among the four laboratories.
160 [7]Includes the data from Balls et al. (1995) using the overall *in vitro* classification based on the majority and/or most severe classification among the four laboratories.

161　**Table II-5.**　**False Negative and False Positive Rates of the ICE Test Method, by**
162　　　　　　　**Chemical Class and Properties of Interest, for the GHS[1] Classification**
163　　　　　　　**System**
164

Category	N[2]	False Positive Rate[3]		False Negative Rate[4]	
		%	No.[5]	%	No.
Overall	144	8	9/114	50	15/30
Chemical Class[6]					
Alcohol	12	50	5/10	50	1/2
Amine/Amidine	5	0	0/2	33	1/3
Carboxylic acid	10	0	0/3	43	3/7
Ester	9	13	1/8	0	0/1
Heterocycle	9	0	0/3	33	2/6
Onium compound	8	0	0/2	33	2/6
Properties of Interest					
Liquids	108	10	9/90	44	8/18
Solids	36	0	0/24	58	7/12
Pesticide	11	0	0/6	60	3/5
Surfactant – Total	21	0	0/12	56	5/9
-nonionic	4	0	0/3	100	1/1
-anionic	2	0	0/1	100	1/1
-cationic	7	0	0/1	33	2/6
pH – Total[7]	20	-	-	40	8/20
- acidic (pH < 7.0)	12	-	-	33	4/12
- basic (pH > 7.0)	8	-	-	50	4/8
Category 1 Subgroup[8]					
- Total	30	-	-	50	15/30
- 4 (CO=4 at any time)	13	-	-	39	5/13
- 3 (severity/persistence)	1	-	-	0	0/1
- 2 (severity)	6	-	-	50	3/6
- 2-4 combined[9]	*20*	-	-	*45*	*9/20*
- 1 (persistence)	10	-	-	70	7/10

165　[1]GHS =- Globally Harmonized System (UN [2003]).
166　[2]N = number of substances.
167　[3]False Negative Rate = the proportion of all positive substances that are falsely identified as negative *in vitro*; [4]False Positive
168　Rate = the proportion of all negative substances that are falsely identified as positive *in vitro*; n = number of substances.
169　[5]Data used to calculate the percentage.
170　[6]Chemical classes included in this table are represented by at least five substances tested in the ICE test method and
171　assignments are based on the MeSH categories (www.nlm.nih.gov/mesh) as defined in **Appendix B**.
172　[7]Total number of GHS Category 1 substances for which pH information was obtained.
173　[8]NICEATM-defined subgroups assigned based on the lesions that drove classification of a GHS Category 1 substance. 1:
174　based on lesions that are persistent; 2: based on lesions that are severe (not including CO=4); 3: based on lesions that are
175　severe (not including CO=4) and persistent; 4: corneal opacity (CO) = 4 at any time.
176　[9]Subcategories 2 to 4 combined to allow for a direct comparison of GHS Category 1 substances classified *in vivo* based on
177　some lesion severity component and those classified based on persistent lesions alone.

178 **3.0   RELIABILITY OF THE ICE TEST METHOD - REANALYSIS**

179

180 **3.2   Reanalysis of ICE Test Method Intralaboratory Repeatability**

181

182 **Table II-7.   Intralaboratory Repeatability of ICE Test Method Endpoints – Prinsen (2000)**

183

Substance (Experiment No.[1])	EU[2] Class[3]	CT[4] (mean[5])	CT (%CV[6])	CS[7] (mean)	CS (%CV)	CO[8] (mean)	CO (%CV)	FR[9] (mean)	FR (%CV)	Index[10] (mean)	Index (%CV)
SP-1 (1)[11]	NI	60	3.3	0.7	346.4	0.3	86.6	0.3	86.6	15	41.6
SP-1 (2)	NI	63.3	3.3	1.7	91.6	0.3	86.6	0.5	0	18.3	39.4
SP-1 (3)	NI	62.3	2.4	2.3	24.7	0.5	0	0	-	12.3	4.7
SP-1 (4)	NI	61.7	0.9	-1.3	-86.6	0	-	0	-	-1.3	-86.6
SP-1 (5)	NI	63.3	0.9	2	0	0	-	0	-	2	0
SP-4 (1)	R36	68.7	3.0	14.3	24.5	3	0	2	0	114.3	3.1
SP-4 (2)	R36	69.3	3.0	13.3	40.0	2	0	2	0	93.3	5.3
SP-4 (3)	R36	75.7	3.3	21	23.8	2.7	21.6	2	0	114.3	14.0
SP-4 (4)	R36	69.7	4.4	14	49.5	2.7	21.6	2	0	107.3	15.1
SP-5 (5)	R36	70	3.8	12.7	27.7	2	0	2	0	92.7	3.8
SU-4 (1)	R36	72	2.4	13.7	18.4	0.7	43.3	1	0	47	16.9
SU-4 (2)	R36	68.7	3.4	14	12.4	0.7	43.3	1	0	47.3	8.5
SU-4 (3)	R36	67.7	6.0	13	15.4	0.7	43.3	1	0	46.3	9.0
SU-4 (4)	R36	66.7	3.5	11	31.5	0.8	34.6	1	0	47.7	10.6
SU-4 (5)	R36	67.7	2.2	9.7	15.8	0.7	43.3	1	0	43	16.3
SU-5 (1)	R41	77.7	1.5	23	24.2	2	0	2	0	103	5.4
SU-5 (2)	R41	74.7	4.7	20.7	19.6	2	0	2	0	100.7	4.0
SU-5 (3)	R41	75.3	6.1	21	9.5	2	0	2	0	101	2.0

Substance (Experiment No.[1])	EU[2] Class[3]	CT[4] (mean[5])	CT (%CV[6])	CS[7] (mean)	CS (%CV)	CO[8] (mean)	CO (%CV)	FR[9] (mean)	FR (%CV)	Index[10] (mean)	Index (%CV)
SU-5 (4)	R41	76.7	2.0	16.3	25.5	1.7	34.6	2	0	89.7	16.4

184 [1]No. = Number.
185 [2]EU = European Union (EU [2001]).
186 [3]Class. = Classification (EU [2001]).
187 [4]CT = Corneal thickness.
188 [5]Mean values calculated with scores from three eyes.
189 [6]%CV = % coefficient of variation.
190 [7]CS = Corneal swelling.
191 [8]CO = Corneal opacity.
192 [9]FR = fluorescein retention.
193 [10]Index = ICE Irritation Index (= CS x [CO x 20] + FR x 20]); No. = number.
194 [11]*In vivo* animal data were not provided for these substances, and therefore the EU classification that was provided by testing laboratory is presented here.

195 **3.3   Reanalysis of ICE Test Method Intralaboratory Reproducibility**
196
197 **Table II-8.   Intralaboratory Reproducibility of ICE Test Method Endpoints – Prinsen (2000)**
198

Substance (Experimental Replicates)	EU[1] Class[2]	CT[3] (mean[4])	CT (%CV[5])	CS[6] (mean)	CS (%CV)	CO[7] (mean)	CO (%CV)	FR[8] (mean)	FR (%CV)	Index[9] (mean)	Index (%CV)
SP-1 (5)[10]	NI	62.1	2.2	1.1	138.7	0.2	95.8	0.2	141.4	9.3	91.8
SP-4 (5)	R36	70.7	4.0	15.1	22.4	2.5	18.1	2	0	104.4	10.3
SU-4 (5)	R36	70.5	6.3	12.3	15.2	0.7	10.6	1	0	46.3	4.1
SU-5 (4)	R41	76.1	1.8	20.2	13.9	1.9	8.7	2	0	98.6	6.1

199 [1]EU = European Union (EU [2001]).
200 [2]Class. = Classification (EU [2001]).
201 [3]CT = Corneal thickness.
202 [4]Mean values calculated with scores from three eyes.
203 [5]%CV = % coefficient of variation.
204 [6]CS = Corneal swelling.
205 [7]CO = Corneal opacity.
206 [8]FR = fluorescein retention.
207 [9]Index = ICE Irritation Index (= CS x [CO x 20] + FR x 20]); No. = number.
208 [10]*In vivo* animal data were not provided for these substances, and therefore the EU classification that was provided by testing laboratory is presented here.

209   **3.4   Reanalysis of ICE Test Method Interlaboratory Reproducibility**
210
211   **Table II-9.   Interlaboratory Variability of Balls et al. (1995) for Substances Classified**
212   **as Ocular Corrosives/Severe Irritants or Nonsevere Irritants/Nonirritants**
213   **Using the GHS[1] Classification System**
214

Classification (*in vivo*/ *in vitro*)[2]	Data Set	Number of Substances	Number of Testing Labs[3]	Substances with 100% Agreement Among Labs	Substances with 75% Agreement Among Labs	Substances with 50% Agreement Among Labs
+/+	New[4]	11	4[3]	7 (64%)	3 (27%)	1 (9%)
	Old[4]	12	4[3]	8 (67%)	3 (25%)	1 (8%)
+/-	New	11	4	9 (82%)	2 (18%)	0 (0%)
	Old	10	4	8 (80%)	2 (20%)	0 (0%)
-/+	New	6	4	1 (17%)	0 (0%)	5 (83%)
	Old	6	4	1 (17%)	0 (0%)	5 (82%)
-/-	New	26	4	22 (85%)	4 (15%)	0 (0%)
	Old	28	4	24 (86%)	4 (14%)	0 (0%)
?/-	New	3	4	3 (100%)	0 (0%)	0 (0%)
	Old	2	4	2 (100%)	0 (0%)	0 (0%)
?/+	New	2	4	2 (100%)	0 (0%)	0 (0%)
	Old	1	4	1 (100%)	0 (0%)	0 (0%)
TOTAL	New	59	4[3]	44 (75%)	9 (15%)	6 (10%)
	Old	59	4[3]	44 (75%)	9 (15%)	6 (10%)

215   [1]GHS = Globally Harmonized System (UN [2003]).
216   [2]A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category 1); a "-"
217   indicates that the substance was assigned an overall classification of nonsevere irritant (Category 2A, 2B) or nonirritant; a
218   "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of
219   effects), a GHS classification could not be made. See **Section II-2.0** for a description of the rules followed to classify the
220   ocular irritancy of test substances tested multiple times *in vitro.*
221   [3]Scores for fluorescein retention and corneal swelling were not provided from one laboratory for one substance
222   (trichloroacetic acid, 30%), and therefore this substance was classified based on results from only three laboratories.
223   [4]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in
224   the draft ICE BRD.
225
226
227   **3.5   ICE Test Method Historical Positive and Negative Control Data - Reanalysis**
228
229   Subsequent to the original analysis, individual eye data were obtained from negative control

230   eyes that could be used to perform a CV analysis on between-experiment values for each of

231   the test method endpoints (i.e., corneal thickness/swelling, corneal opacity, fluorescein

232   retention) along with the ICE Irritation Index for each test substance. This analysis revealed

233   that responses in the negative control eye remain relatively consistent.

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244   **SECTION III**

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246   **BOVINE CORNEAL OPACITY AND**

247   **PERMEABILITY (BCOP) TEST METHOD**

248   **ACCURACY AND RELIABILITY REANALYSIS**

249   **SUMMARY**

250   **1.0   INTRODUCTION**

251

252   **Table III-1.   Summary of BCOP Database Changes**

253

Data Source	Data Base	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
			EPA[1]	EU[2]	GHS[3]	
			Cat[4] I/Total[5]	R41/Total	Cat 1/Total	
Gautheron (1994)	New[6]	51	7/48	7/48	7/47	Additional in vivo animal data were received subsequent to the original analysis that allowed for classification according to all three classification systems.
	Old[6]	51	6/12	8/51	7/13	
Balls et al. (1995)	New	59	18/53	19/50	22/54	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient in vivo rabbit eye test data for classification (See **Appendix A**).
	Old	59	20/55	21/59	22/57	
Swanson et al. (1995)	New	20	6/8	6/9	6/8	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient in vivo rabbit eye test data for classification (See **Appendix A**). The increase in the number of corrosive/severe irritants is due to the reclassification of substances.
	Old	20	6/9	5/9	6/9	
Casterton (1996)	New	97	27/56	25/54	27/55	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient in vivo rabbit eye test data for classification (See **Appendix A**). The increase in the number of corrosive/severe irritants is due to the reclassification of substances.
	Old	97	26/55	24/60	26/56	

Data Source	Data Base	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
			EPA[1]	EU[2]	GHS[3]	
			Cat[4] I/Total[5]	R41/Total	Cat 1/Total	
Gettings (1996)	New	25	10/25	8/23	8/23	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient in vivo rabbit eye test data for classification (See **Appendix A**). The increase in the number of corrosive/severe irritants is due to the reclassification of substances.
	Old	25	10/25	6/25	8/25	
Southee (1998)	New	16	5/14	6/14	7/15	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient in vivo rabbit eye test data for classification (See **Appendix A**). The change in the number of corrosive/severe irritants is due to the reclassification of substances.
	Old	16	6/14	5/15	6/14	
Swanson and Harbell (2000)	New	13	4/9	1/9	1/9	
	Old	13	4/9	1/9	1/9	
Bailey (2004)	New	16	1/13	3/13	3/14	The decrease in the total number of usable substances is due to excluding substances from consideration due to insufficient in vivo rabbit eye test data for classification (See **Appendix A**). The change in the number of corrosive/severe irritants is due to the reclassification of substances.
	Old	16	3/16	3/16	3/16	

254 [1]EPA = U.S. Environmental Protection Agency (EPA [1996]).
255 [2]EU = European Union (EU [2001]).
256 [3]GHS = Globally Harmonized System (UN [2003]).
257 [4]Cat = category.
258 [5]First number (before forward slash) refers to the number of substances in each study that were classified as a severe irritant according to each classification system (EPA, EU,
259 and GHS). The second number (after the forward slash) refers to the number of substances in were classified, based on animal data, for each classification system (EPA, EU,
260 GHS).
261 [6]New = accuracy statistics based on revised analysis; New = accuracy statistics based on the previous analysis included in the draft BCOP BRD.

262 **2.0   ACCURACY OF THE BCOP TEST METHOD – REANALYSIS**
263

264 **Table III-2.   Evaluation of the Performance of the BCOP Test Method In Predicting Ocular Corrosives and Severe Irritants**
265 **Compared to the In Vivo Rabbit Eye Test Method, as Defined by the GHS[1] Classification System, by Study and**
266 **Overall**
267

Data Source	Anal.[2]	N[3]	Accuracy		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
			%	No.[4]	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Gautheron et al. 1994 (new)[5]	IVIS	47/52	74[6]	35/47	71	5/7	75	30/40	33	5/15	94	30/32	25	11/40	29	2/7
Gautheron et al. 1994 (old)[5]	IVIS	13/52	77[6]	10/13	71	5/7	83	5/6	83	5/6	71	5/7	17	1/6	29	2/7
Balls et al. 1995 (new)[7]	IVIS	54/59	70[6]	38/54	77	17/22	66	21/32	61	17/28	81	21/26	34	11/32	23	5/22
Balls et al. 1995 (old)	IVIS	57/59	70[6]	40/57	77	17/22	66	23/35	59	17/29	82	23/28	34	12/35	23	5/22
Swanson et al. 1995 (new)	IVIS	8/20	100	8/8	100	6/6	100	2/2	100	6/6	100	2/2	0	0/2	0	0/6
Swanson et al. 1995 (old)	IVIS	9/20	89	8/9	100	6/6	67	2/3	86	6/7	100	2/2	33	1/3	0	0/6
Gettings et al. 1996 (new)	Perm	23/25	87	20/23	75	6/8	93	14/15	86	6/7	88	14/16	7	1/15	25	2/8
Gettings et al. 1996 (old)	Perm	25/25	88	22/25	75	6/8	94	16/17	86	6/7	89	16/18	6	1/17	25	2/8
Casterton et al. 1996 (new)	O/P	55/97	67	37/55	48	13/27	86	24/48	76	13/17	63	24/38	14	4/28	52	14/27
Casterton et al. 1996 (old)	O/P	56/97	66	37/56	46	12/26	83	25/30	71	12/17	64	25/39	17	5/30	54	14/26
Southee 1998 (new)	IVIS	15/16	73	11/15	57	4/7	88	7/8	80	4/5	70	7/10	12	1/8	43	3/7
Southee 1998 (old)	IVIS	14/16	64[6]	9/14	50	3/6	75	6/8	40	2/5	67	6/9	25	2/8	50	3/6
Swanson & Harbell 2000 (new)	IVIS	9/13	78	7/9	100	1/1	75	6/8	33	1/3	100	6/6	25	2/8	0	0/1
Swanson & Harbell 2000 (old)	IVIS	9/13	78	7/9	100	1/1	75	6/8	33	1/3	100	6/6	25	2/8	0	0/1
Bailey et al. 2004 (new)	IVIS	14/16	93	13/14	67	2/3	100	11/11	100	2/2	92	11/12	0	0/11	33	1/3
Bailey et al. 2004 (old)	IVIS	16/16	94	15/16	67	2/3	100	13/13	100	2/2	93	13/14	0	0/13	33	1/3
Entire Data Set[8] (new)		147/203	81	119/147	84	36/43	80	83/104	63	36/57	92	83/90	20	21/104	16	7/43
Entire Data Set (old)		129/200	79	95/120	76	32/42	81	63/78	69	34/49	86	61/71	19	15/78	24	10/42

268　[1]GHS = Globally Harmonized System (UN [2003]).
269　[2]Anal. = analytical method used to transform the sample data into BCOP classification. IVIS = In Vitro Irritancy Score developed by Gautheron et al. (1994).
270　Perm = Permeability value only used to classify in vitro ocular irritancy in the BCOP assay; an OD_{490} value >0.600 was considered a severe irritant. O/P =
271　irritation class based on the endpoint (opacity or permeability) with the highest score for its respective range (Casterton et al. [1996]).
272　[3]N = number of substances included in this analysis/the total number of substances evaluated in the study.
273　[4]Data used to calculate the percentage.
274　[5]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in the draft BCOP BRD.
275　[6]Performance calculated using the overall in vitro classification based on the majority and/or most severe classification among the multiple testing laboratories
276　and tests (for substances tested multiple times in a laboratory).
277　[7]The test substance 1% benzalkonium chloride was tested in two different in vivo studies, producing discordant results with respect to GHS classification; the
278　analysis was performed using the Category 1 classification.
279　[8]Data from Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Gettings et al. (1996), Southee (1998), Swanson and Harbell (2000), and Bailey et
280　al. (2004) were pooled together and an overall in vitro classification was assigned for each test substance based on the majority and/or most severe classification
281　obtained across tests and testing laboratories. Data from Casterton et al. (1996) were not included in this analysis since the protocol used to generate BCOP data
282　differed considerably from the other studies (e.g., a spectrophotometer was used to measure opacity instead of an opacitometer, and solids were applied neat
283　instead of as a 20% solution or suspension).

284 **Table III-5.   False Negative and False Positive Rates of the BCOP Test Method,**
285 **by Chemical Class and Properties of Interest, for the GHS[1]**
286 **Classification System**
287

Category	N[2]	False Positive Rate[3]		False Negative Rate[4]	
		%	No.[5]	%	No.
Overall	147	20	21/104	16	7/43
Chemical Class[6]					
Alcohol	21	50	9/18	67	2/3
Amine/Amidine	8	0	0/4	0	0/4
Carboxylic acid	16	33	3/9	14	1/7
Ester	12	12	1/8	0	0/4
Ether/Polyether	6	0	0/5	0	0/1
Heterocycle	12	33	2/6	17	1/6
Hydrocarbon	11	9	1/11	-	0/0
Inorganic salt	5	0	0/3	0	0/2
Ketone	9	33	3/9	-	0/0
Onium compound	11	0	0/3	0	0/8
Properties of Interest					
Liquids	93	26	18/69	4	1/24
Solids	34	10	2/20	43	6/14
Pesticide	8	33	1/3	40	2/5
Surfactant – Total[7]	35	5	1/21	7	1/14
-nonionic	5	0	0/4	0	0/1
-anionic	3	0	0/2	100	1/1
-cationic	6	0	0/1	0	0/7
pH – Total[8]	24	-	-	21	5/24
- acidic (pH < 7.0)	11	-	-	18	2/11
- basic (pH > 7.0)	13	-	-	23	3/13
Category 1 Subgroup[9] - Total	38	-	-	18	7/38
- 4 (CO=4 at any time)	20	-	-	15	3/20
- 3 (severity/persistence)	1	-	-	0	0/1
- 2 (severity)	4	-	-	25	1/4
- 2-4 combined[10]	25	-	-	17	4/24
- 1 (persistence)	13	-	-	23	3/13

288 [1]GHS = Globally Harmonized System (UN [2003]).
289 [2]N = number of substances.
290 [3]False Positive Rate = the proportion of all negative substances that are falsely identified as positive in vitro.
291 [4]False Negative Rate = the proportion of all positive substances that are falsely identified as negative in
292 vitro.
293 [5]Data used to calculate the percentage.
294 [6]Chemical classes included in this table are represented by at least five substances tested in the BCOP test
295 method and assignments are based on the MeSH categories (www.nlm.nih.gov/mesh) as defined in
296 **Appendix B**.
297 [7]Combines single chemicals labeled as surfactants along with surfactant-containing formulations.
298 [8]Total number of GHS Category 1 substances for which pH information was obtained.
299 [9]NICEATM-defined subgroups assigned based on the lesions that drove classification of a GHS Category 1
300 substance. 1: based on lesions that are persistent; 2: based on lesions that are severe (not including Corneal
301 Opacity [CO]=4); 3: based on lesions that are severe (not including CO=4) and persistent; 4: CO = 4 at any
302 time.
303 [10]Subcategories 2 to 4 combined to allow for a direct comparison of GHS Category 1 substances classified in
304 vivo based on some lesion severity component and those classified based on persistent lesions alone.
305

305 **3.0   RELIABILITY OF THE BCOP TEST METHOD - REANALYSIS**

306

307 **3.2   Reanalysis of BCOP Test Method Intralaboratory Repeatability**

308

309 The updated information received subsequent to the release of the draft BCOP BRD did

310 not affect the analyses of intralaboratory repeatability and therefore these are not

311 discussed again here (see the draft BCOP BRD, published November 1, 2004).

312

313 **3.3   Reanalysis of BCOP Test Method Intralaboratory Reproducibility**

314

315 The updated information received subsequent to the release of the draft BCOP BRD did

316 not affect the analyses of intralaboratory reproducibility and therefore these are not

317 discussed again here (see the draft BCOP BRD, November 1, 2004).

318

319

320 **3.4   Reanalysis of BCOP Test Method Interlaboratory Reproducibility**

321

322 **Table III-7.   Evaluation of the Reliability of the BCOP Test Method in Predicting Ocular Corrosives and Severe Irritants as**
323 **Defined by the GHS[1] Classification System, by Study**
324

Report	Classification (In Vivo/In Vitro)[2]	No. of Testing Labs	N[3]	Substances with 100% Agreement among Labs[4]	Substances with 91-92% Agreement among Labs	Substances with 82-83% Agreement among Labs	Substances with 80% Agreement among Labs	Substances with 73% Agreement among Labs	Substances with 64-67% Agreement among Labs	Substances with 58-60% Agreement among Labs	Substances with ≤ 55% Agreement among Labs
	+/+ (new)[5]	5	17	13 (76%)			3 (18%)			1 (6%)	
	+/+ (old)[5]	5	17	14 (82%			2 (12%)			1 (6%)	
	+/- (new)	5	5	3 (60%)			1 (20%)			1 (20%)	
	+/- (old)	5	5	3 (60%)			1 (20%)			1 (20%)	
	-/+ (new)	5	11	4 (36%)			4 (36%)			3 (27%)	
	-/+ (old)	5	12	4 (33%)			5 (42%)			3 (25%)	
Balls et al.	-/- (new)	5	21	16 (76%)			2 (10%)			3 (14%)	
(1995)	-/- (old)	5	23	17 (74%)			2 (9%)			4 (17%)	
	?/- (new)	5	4	3 (75%)						1 (25%)	
	?/- (old)	5	2	2 (100%)						0 (0%)	
	?/+ (new)	5	2	2 (100%)							
	?/+ (old)	5	1	1 (100%)							
	Total (new)		60	41 (68%)			10 (17%)			9 (15%)	
	Total (old)		60	41 (68%)			10 (17%)			9 (15%)	
Gautheron et al. (1994)	+/+ (new)	11	5	3 (60%)	1 (10%)						1 (10%)
		12	1	1(100%)							
	+/+ (old)	11	4	2 (50%)	1 (25%)						1 (25%)
		12	1	1 (100%)							
	+/- (new)	11	1			1(100%)					
		12	1	1(100%)							
	+/- (old)	11	1			1 (100%)					
		12	1	1 (100%)							
	-/+ (new)	11	4	2 (50%)		1 (25%)		1 (25%)			
		12	5	2 (40%)	1 (20%)						2 (40%)
	-/+ (old)	11	1						1 (100%)		
	-/- (new)	11	2			1 (50%)				1 (50%)	
		12	28	23 (81%)	1 (4%)	3 (11%)				1 (4%)	
	-/- (old)	11	4	3 (75%)			1 (25%)				

25

Report	Classification (In Vivo/In Vitro)[2]	No. of Testing Labs	N[3]	Substances with 100% Agreement among Labs[4]	Substances with 91-92% Agreement among Labs	Substances with 82-83% Agreement among Labs	Substances with 80% Agreement among Labs	Substances with 73% Agreement among Labs	Substances with 64-67% Agreement among Labs	Substances with 58-60% Agreement among Labs	Substances with ≤ 55% Agreement among Labs
		12	1			1 (100%)					
	?/- (new)	11	1					1 (100%)			
		12	1	1 (100%)							
	?/- (old)	11	11	8 (73%)		2 (18%)		1 (9%)			
		12	16	15 (94%)	1 (6%)						
	?/+ (new)	11	3	1 (33%)	1 (33%)				1 (33%)		
	?/+ (old)	11	7	4 (57%)	1 (14%)	1 (14%)		1 (14%)			
		12	4	2 (50%)	1 (25%)					1 (25%)	
	Total (new)		52	34 (65%)	3 (6%)	7 (13%)		2 (4%)	3 (6%)		3 (6%)
	Total (old)		51	36 (71%)	3 (6%)	6 (12%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
	+/+ (new)	3	4	4 (100%)							
	+/+ (old)	3	3	3 (100%)							
	+/- (new)	3	3	3 (100%)							
	+/- (old)	3	3	3 (100%)							
	-/+ (new)	3	1	1 (100%)							
	-/+ (old)	3	2	2 (100%)							
Southee (1998)	-/- (new)	3	7	6 (86%)					1 (14%)		
	-/- (old)	3	6	5 (83%)					1 (17%)		
	?/- (new)	3	1	1 (100%)							
	?/- (old)	3	2	2 (100%							
	?/+ (new)	-	0								
	?/+ (old)	-	0								
	Total (new)		16	15 (94%)					1 (6%)		
	Total (old)		16	15 (94%)					1 (6%)		

325 [1]GHS = Globally Harmonized System (UN [2003]).

326 [2]A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category 1); a "-" indicates that the substance was
327 assigned an overall classification of nonsevere irritant (Category 2A, 2B) or nonirritant; a "?" indicates that, due to the lack of appropriate in vivo data (e.g.,
328 studies were terminated too early to assess reversibility of effects; insufficient dose volume), a GHS classification could not be made. See **Section 2.0** for a
329 description of the rules followed to classify the ocular irritancy of test substances tested multiple times in vitro.
330 [3]N = number of substances.
331 [4]Number in parentheses indicates percentage of tested chemicals.
332 [5]New = accuracy statistics based on revised analysis; Old = accuracy statistics based on the previous analysis included in the draft BCOP BRD.

333   **3.5   BCOP Test Method Historical Positive and Negative Control Data -**
334   **Reanalysis**
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336   An example of historical data for positive controls was provided by IIVS (current as of July
337   22, 2004), and is provided in the draft BCOP BRD (November 1, 2004).

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SECTION IV

349

HEN'S EGG TEST - CHORIOALLANTOIC

MEMBRANE (HET-CAM) TEST METHOD

ACCURACY AND RELIABILITY REANALYSIS

SUMMARY

354

355   **1.0   INTRODUCTION**
356
357   **Table IV-1.   Summary of HET-CAM Database Changes**
358

Data Source	Data Set	Analysis Method	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
				EPA[1]	EU[2]	GHS[3]	
				Cat I/Total	R41/Total	Cat 1/Total	
Bagley et al. (1992)	New[4]	IS(A)[5]	32	0/2[6]	0/2	0/2	
	Old[4]	IS(A)	32	0/3	0/3	0/3	
Balls et al. (1995)	New	Q-Score[5] S-Score[5]	59	14/45 9/15	13/39 4/14	12/43 4/16	The decrease, where present, in the total number of usable substances is due to excluding substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A**). The increase, where present, in the number of corrosives and severe irritants is due to reclassification of substances.
	Old	Q-Score S-Score	59	10/40 2/12	14/48 4/19	15/45 4/17	
CEC (1991)	New	IS(B)[5]		-	15/21	-	Data previously described in an Addendum to the draft HET-CAM BRD which was released to the public on November 16, 2004. The decrease, where present, in the total number of usable substances is due to excluding substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A**).
	Old	IS(B)		-	21/21	-	
Gettings et al. (1991)	New	IS(B)	9	3/9	3/8	3/9	The decrease, where present, in the total number of usable substances is due to excluding substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A**). The increase, where present, in the number of corrosives and severe irritants is due to reclassification of substances.
	Old	IS(B)	9	3/9	2/9	3/9	

Data Source	Data Set	Analysis Method	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
				EPA[1]	EU[2]	GHS[3]	
				Cat I/Total	R41/Total	Cat 1/Total	
Gettings et al. (1994)	New	IS(A) IS(B)	18	1/18 1/18	1/18 1/18	1/18 1/18	
	Old	IS(A) IS(B)	18	1/18 1/18	1/18 1/18	1/18 1/18	
Gettings et al. (1996)	New	IS(A) IS(B)	25	3/25 9/25	3/23 8/23	3/23 8/23	The decrease, where present, in the total number of usable substances reflects the exclusion of substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A**). The increase, where present, in the number of corrosives and severe irritants is due to reclassification of substances.
	Old	IS(A) IS(B)	25	3/25 9/25	1/25 6/25	3/23 8/23	
Gilleron et al. (1996)	New	IS(B)		-	2/43	-	Data previously described in Section 9.0 of the draft HET-CAM BRD. Data were included in the reanalysis for the ability of the test method to accurately classify test substances according to the EU classification system.
	Old	IS(B)	0	-	-	-	
Gilleron et al. (1997)	New	IS(B)	60	16/53	16/48	19/54	Data previously described in Section 9.0 of the draft HET-CAM BRD. Data were included in the reanalysis for the ability of the test method to accurately classify test substances according to the GHS, EPA, and EU classification system.
	Old	IS(B)	0	-	-	-	
Hagino et al. (1999)	New	IS(A)	17	7/15	7/15	8/12	The decrease, where present, in the total number of usable substances reflects the exclusion of substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A**). The increase, where present, in the number of corrosives and severe irritants is due to reclassification of substances.
	Old	IS(A)	17	6/14	7/17	8/16	

Data Source	Data Set	Analysis Method	Number of Available Substances	Number of Acceptable Substances by Ocular Irritancy Classification System			Comments
				EPA[1]	EU[2]	GHS[3]	
				Cat I/Total	R41/Total	Cat 1/Total	
Kojima et al. (1995)	New	IS(A)	24	2/5	2/4	2/5	The decrease, where present, in the total number of usable substances is due to excluding substances from consideration due to insufficient rabbit eye test data for classification (See **Appendix A)**.
	Old	IS(A)	24	2/5	2/5	2/5	
Spielmann et al. (1996)	New	mtc10[5]	142	-	25/142	-	
	New	mtc10	189	-	30/189	-	
	New	IS(B)-10[5] IS(B)-100[5]	120 120	11/73 13/70	14/71 16/69	19/77 21/75	Previous ocular irritancy calls only available for EU classification system. Additional *in vivo* and *in vitro* data received which allowed for an accuracy evaluation when compared to all three classification systems.
	Old	IS(B)-10 IS(B)-100	0 0	- -	- -	- -	
Vinardell and Macián (1994)	New	IS(B)	13	0/2	0/2	0/2	
	Old	IS(B)	13	0/2	0/2	0/2	

359 [1]EPA = U.S. Environmental Protection Agency (EPA [1996]).
360 [2]EU = European Union (EU [2001]).
361 [3]GHS = Globally Harmonized System (UN [2003]).
362 [4]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in the draft HET-CAM BRD.
363 [5]IS(A) = method described in Luepke (1985); IS(B), IS(B)-10, and IS(B)-100 = method described in Kalweit et al. (1987); Q = Q-Score, method described in
364 Balls et al. (1995); S = S-Score, method described in Balls et al. (1995); mtc10 = mean time to coagulation after administration of a 10% solution, method
365 described in Spielmann et al. (1996).
366 [6]First number (before forward slash) refers to the number of substances in each study that were classified as a severe irritant according to each classification
367 system (EPA, EU, and GHS). The second number (after the forward slash) refers to the number of substances in were classified, based on animal data, for each
368 classification system (EPA, EU, GHS).

369 **2.0   ACCURACY OF THE HET-CAM TEST METHOD - REANALYSIS**
370
371 **Table IV-3.   Evaluation of the Performance of the HET-CAM Test Method in Predicting Ocular Corrosives and Severe**
372 **Irritants Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS[1] Classification System, by**
373 **HET-CAM Analysis Method**
374

Analysis Method[2]	Data Set	N[3]	Accuracy		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
			%	No.[4]	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
IS(A)-100[5]	New[6]	20	85	17/20	100	2/2	83	15/18	40	2/5	100	15/15	17	3/18	0	0/2
IS(A)-10[5]	New	24	50	12/24	25	4/12	100	8/8	100	4/4	40	8/20	0	0/8	75	12/16
IS(A)	New	64	66	42/64	52	14/29	77	27/35	65	15/23	66	27/41	23	8/35	48	15/29
IS(A)	Old[6]	61	75	46/61	67	12/18	79	34/43	57	12/21	85	34/40	21	9/43	33	6/18
IS(B)-100[5] (Entire database)	New	143	53	76/143	85	35/41	40	41/102	36	35/96	87	41/47	60	61/102	15	6/41
IS(B)-100[5] (Spielmann et al. 1996)	New	75	55	41/75	88	21/24	39	20/51	40	21/31	87	20/23	61	31/51	13	3/24
IS(B)-10[5](Entire database)	New	101	68	69/101	70	28/40	67	41/61	58	28/48	77	41/53	33	20/61	30	12/40
IS(B)-10[4] (Spielmann et al. 1996)	New	77	68	52/77	79	19/24	62	33/53	49	19/39	87	33/38	38	20/53	21	5/24
IS(B)	New	107	57	61/107	76	32/42	45	29/65	47	32/68	74	29/39	55	36/65	24	10/42
IS(B)	Old	52	85	44/52	100	12/12	80	32/40	60	12/20	100	32/32	20	8/40	0	0/12
Q-Score	New	43	63	27/43	100	12/12	43	12/28	48	15/31	100	12/12	57	16/28	0	0/12
Q-Score	Old	45	63	28/45	100	15/15	43	13/30	47	15/32	100	13/13	57	17/30	0	0/15
S-Score	New	16	44	7/16	36	4/11	60	3/5	67	4/6	30	3/10	40	2/5	64	7/11
S-Score	Old	17	47	8/17	36	4/11	67	4/6	67	4/6	36	4/11	33	2/6	64	7/11

375 [1]GHS = Globally Harmonized System (UN [2003]).
376 [2]IS(A), IS(A)-10, IS(A)-100 = method described in Luepke (1985); IS(B), IS(B)-10, IS(B)-100 = method described in Kalweit et al. (1987); Q = Q-Score,
377 method described in Balls et al. (1995); S = S-Score, method described in Balls et al. (1995).
378 [3]N = number of substances evaluated in each study.

379   [4]Data used to calculate the percentage.
380   [5]The analysis compares the ability of the specified concentration tested *in vitro* (IS(A)-10 represents the 10% concentration tested *in vitro*) to predict the effect
381   produced by the undiluted test substance tested *in vivo*.
382   [6]New = accuracy statistics based on the revised analysis; Old = accuracy statistics based on the previous analysis included in the draft HET-CAM BRD.

Table IV-8. **False Negative and False Positive Rates of the HET-CAM Test Method, by Chemical Class and Properties of Interest, for the GHS[1] Classification System**

Category	N[2]	False Positive Rate[3]		False Negative Rate[3]	
		%	No.	%	No.
Overall IS(B)-10 (Entire database)	101	33	20/61	30	12/40
Overall IS(B)-100 (Entire database)	143	60	61/102	15	6/41
Chemical Class[4]-IS(B)-10					
Alcohol	17	90	9/10	25	2/7
Amine	7	60	3/5	50	1/2
Ether	14	50	5/10	50	2/4
Formulation	24	0	0/8	44	7/16
Heterocycle	6	83	5/6	-	-
Organic salt	7	57	4/7	-	-
Chemical Class[4]-IS(B)-100					
Alcohol	20	91	10/11	11	1/9
Aldehyde	6	80	4/5	0	0/1
Amine	10	83	5/6	50	2/4
Ester	14	83	10/12	0	0/2
Ether	20	60	9/15	20	1/5
Formulation	51	19	6/31	35	7/13
Heterocycle	10	75	6/8	-	-
Inorganic salt	5	100	2/2	0	0/3
Ketone	6	67	4/6	-	-
Onium	7	100	2/2	0	0/5
Organic salt	8	88	7/8	-	-
Properties of Interest					
Physical Form: IS(B)-10					
Liquid	101	33	20/61	30	10/40
Solid	-	-	-	-	-
Physical Form: IS(B)-100					
Liquid	63	67	36/54	0	0/9
Solid	43	67	16/24	26	5/19
Unknown	37	38	9/24	8	1/13
Surfactant – Total IS(B)-100	3	66	2/3	-	-
-nonionic	3	66	2/3	-	-
-anionic	0	-	-	-	-
-cationic	0	-	-	-	-
Surfactant-Based Formulation – IS(B)-10	24	0	0/8	44	7/16
pH – IS(B)-10[5]	35	58	11/19	13	2/16
- acidic (pH < 7.0)	24	50	7/14	20	2/10
- basic (pH > 7.0)	11	80	4/5	0	0/6
pH – IS(B)-100[5]	35	68	13/19	13	2/16
- acidic (pH < 7.0)	23	69	9/13	10	1/10
- basic (pH > 7.0)	12	67	4/6	17	1/6

Category	N[2]	False Positive Rate[3]		False Negative Rate[3]	
		%	No.	%	No.
Category 1 Subgroup- IS(B)-10[6]					
- Total	40	-	-	30	12/40
- 4 (CO=4 at any time)	13	-	-	15	2/13
- 3 (severity/persistence)	0	-	-	-	-
- 2 (severity)	0	-	-	-	-
- *2-4 combined*[7]	*13*	-	-	15	2/11
- 1 (persistence)	27	-	-	37	10/27
Category 1 Subgroup- IS(B)-100[6]					
- Total	37	-	-	11	4/37
- 4 (CO=4 at any time)	19	-	-	11	2/19
- 3 (severity/persistence)	2	-	-	0	0/2
- 2 (severity)	2	-	-	0	0/2
- *2-4 combined*[7]	*23*	-	-	9	2/23
- 1 (persistence)	18	-	-	11	2/18

387 [1]GHS = Globally Harmonized System (UN [2003]).
388 [2]N=number of substances
389 [3]False Positive Rate = the proportion of all negative substances that are falsely identified as positive in vitro; n =
390 number of substances; False Negative Rate = the proportion of all positive substances that are falsely identified as
391 negative in vitro.
392 [4]Chemical classes included in this table are represented by at least five substances tested in the HET-CAM test
393 method and assignments are based on the MeSH categories (www.nlm.nih.gov/mesh). See **Appendix B**.
394 [5]Total number of GHS Category 1 substances for which pH information was obtained.
395 [6]NICEATM-defined subgroups assigned based on the lesions that drove classification of a GHS Category 1
396 substance. 1: based on lesions that are persistent; 2: based on lesions that are severe (not including Corneal Opacity
397 [CO]=4); 3: based on lesions that are severe (not including CO=4) and persistent; 4: CO = 4 at any time.
398 [7]Subcategories 2 to 4 combined to allow for a direct comparison of GHS Category 1 substances classified *in vivo*
399 based on some lesion severity component and those classified based on persistent lesions alone.
400

400 **3.0   RELIABILITY OF THE HET-CAM TEST METHOD - REANALYSIS**

401

402 **3.2   Reanalysis of HET-CAM Test Method Intralaboratory Repeatability**

403

404 **Tables IV-11/12.   Intralaboratory Repeatability Evaluation for Substances Tested Using**
405 **the IS(B) Analysis Method (*Summary of Tables IV-11 and IV-12 in***
406 ***HET-CAM Addendum*)**

407

Study		Hemorrhage	Lysis	Coagulation	Overall IS(B) Score
Gilleron et al. (1996)	Mean (SD) for All Substances[a]	1.64 (1.93)	2.68 (2.88)	3.59 (3.44)	7.92 (5.84)
	%CV for All Substances[b]	**117.56**	**107.52**	**95.69**	**73.74**
	Mean (SD) Excluding Nine Substances[a, c]	1.63 (1.90)	1.87 (2.57)	2.83 (3.25)	6.33 (5.43)
	%CV Excluding Nine Substances[b, c]	**116.13**	**137.49**	**115.07**	**85.84**
Gilleron et al. (1997)	Mean (SD) for All Substances[a]	1.94 (2.12)	5.60 (2.31)	6.42 (2.68)	13.96 (4.89)
	%CV for All Substances[b]	**109.10**	**41.24**	**41.78**	**34.99**
	Mean (SD) Excluding Four Substances[a, c]	2.07 (2.16)	5.75 (2.19)	6.60 (2.49)	14.42 (4.48)
	%CV Excluding Four Substances[b, c]	**104.43**	**38.04**	**37.78**	**31.05**

408 Abbreviations: CV = coefficient of variation, SD = standard deviation.
409 [a] Mean calculated using the values from the mean of 3 eggs tested for each substance for each endpoint
410 and the Overall IS(B) Score. SD was based on the values in these individual columns.
411 [b] To avoid eliminating data for which the %CV could not be calculated (i.e., where the mean and SD both
412 equaled 0), the %CV values were calculated using the mean and SD calculated as described in footnote a.
413 [c] For some compounds (nine compounds in Gilleron et al. (1996) and four compounds in Gilleron et al.
414 (1997)) the data used in the publication could not be traced in detail by the authors. Therefore, substitute
415 test data for these substances were provided. The results provided exclude these substances.
416

416   **3.3   Reanalysis of HET-CAM Test Method Intralaboratory Reproducibility**

417

418   **Tables IV-13/14.   Intralaboratory Reproducibility Evaluation for Substances Tested**
419   **Using the IS(B) Analysis Method (*Summary of Tables IV-13 and IV-14***
420   ***in HET-CAM Addendum*)**

421

Study		Hemorrhage	Lysis	Coagulation	Overall IS(B) Score
Gilleron et al. (1996)	Mean (SD) for All Substances[a]	1.60 (1.70)	2.51 (2.28)	3.40 (2.89)	7.51 (5.28)
	%CV for All Substances[b]	**106.43**	**91.00**	**84.89**	**70.35**
	Mean (SD) Excluding Nine Substances[a, c]	1.63 (1.71)	1.87 (1.98)	2.83 (2.73)	6.33 (5.06)
	%CV Excluding Nine Substances[b, c]	**104.49**	**106.22**	**96.63**	**79.92**
Gilleron et al. (1997)	Mean (SD) for All Substances[a]	197 (2.04)	5.64 (2.14)	6.46 (2.44)	14.07 (4.62)
	%CV for All Substances[b]	**103.34**	**37.92**	**37.80**	**32.86**
	Mean (SD) Excluding Four Substances[a, c]	2.07 (2.07)	5.75 (2.06)	6.60 (2.28)	14.42 (4.31)
	%CV Excluding Four Substances[b, c]	**100.01**	**35.00**	**34.54**	**29.87**

422   Abbreviations: CV = coefficient of variation, SD = standard deviation.
423   [a] Mean was calculated using the values from the mean of 3 eggs tested for each substance for each
424   endpoint and the Overall IS(B) Score. The SD was calculated based on the values in these individual
425   columns.
426   [b] To avoid eliminating data for which the %CV could not be calculated (i.e., where the mean and SD both
427   equaled 0), the %CV values were calculated using the mean and SD calculated as described in footnote a.
428   [c] For some compounds (nine compounds in Gilleron et al. (1996) and four compounds in Gilleron et al.
429   (1997)) the data used in the publication could not be traced in detail by the authors. Therefore, substitute
430   test data for these substances were provided. The results provided exclude these substances.
431

432 **3.4   Reanalysis of HET-CAM Test Method Interlaboratory Reproducibility**
433
434 **Table IV-15. Evaluation of the Reliability of the HET-CAM Test Method In Predicting Ocular Corrosives and Severe**
435   **Irritants as Defined by the GHS[1] Classification System, by Study**
436

Report	Anal[2]	Classification (In Vivo/In Vitro)[3]	# of Labs	N[4]	Substances with 100% Agreement among Labs	Substances with 80% Agreement among Labs	Substances with 75% Agreement among Labs	Substances with 66% Agreement among Labs	Substances with 60% Agreement among Labs	Substances with ≤50% Agreement among Labs
Balls et al. (1995)	Q	+/+	2 4	4 11	3 (75%)[5] 6 (55%)	-	- 4 (36%)	-	-	1 (25%) 1 (9%)
		+/-	-	-	-	-	-	-	-	-
		-/+	4	16	4 (25%)	-	9 (56%)	-	-	3 (19%)
		-/-	2 4	1 11	1 (100%) 4 (36%)	-	- 7 (64%)	-	-	-
		?/-	2	1	1 (100%)	-	-	-	-	-
		?/+	3 4	1 2	1 (100%) 1 (50%)	-	- 1 (50%)	-	-	-
		Total	2-4	47	21 (45%)	-	21 (45%)	-	-	5 (10%)
Balls et al. (1995)	S	+/+	2	4	4 (100%)	-	-	-	-	-
		+/-	2 3 4	1 4 2	1 (100%) 2 (50%) 2 (100%)	-	-	- 2 (50%) -	-	-
		-/+	2 4	1 1	-	-	-	-	-	1 (100%) 1 (100%)
		-/-	3 4	1 2	1 (100%) 2 (100%)	-	-	-	-	-
		?/-	3	1	-	-	-	1 (100%)	-	-
		?/+	2	2	1 (50%)	-	-	-	-	1 (50%)
		Total	2-4	19	13 (68%)	-	-	3 (16%)	-	3 (16%)
Spielmann et al. (1996)	IS(B)-10	+/+	2 3	18 1	16 (89%) -	- -	- -	- 1 (100%)	- -	2 (11%) -
		+/-	2 3	4 1	4 (100%) -	- -	- -	- 1 (100%)	- -	- -
		-/+	2	16	7 (44%)	-	-	-	-	9 (56%)

Report	Anal[2]	Classification (In Vivo/In Vitro)[3]	# of Labs	N[4]	Substances with 100% Agreement among Labs	Substances with 80% Agreement among Labs	Substances with 75% Agreement among Labs	Substances with 66% Agreement among Labs	Substances with 60% Agreement among Labs	Substances with ≤50% Agreement among Labs
			3	2	1 (50%)	-	-	-	-	1 (50%)
		-/-	2	31	30 (97%)	-	-	-	-	1 (3%)
			3	2	1 (50%)	-	-	1 (50%)	-	-
		?/-	2	10	10 (100%)	-	-	-	-	-
			3	2	1 (50%)	-	-	1 (50%)	-	-
		?/+	2	16	14 (88%)	-	-	-	-	2 (11%)
			3	4	1 (25%)	-	-	2 (50%)	-	1 (25%)
		Total		107	85 (79%)			5 (5%)		16 (15%)
Spielmann et al. (1996)	IS(B) -100	+/+	2	17	16 (94%)	-	-	-	-	1 (6%)
			3	2	1 (50%)	-	-	1 (50%)	-	-
		+/-	2	2	2 (100%)	-	-	-	-	-
		-/+	2	27	20 (74%)	-	-	-	-	7 (26%)
			3	4	1 (25%)	-	-	3 (75%)	-	-
		-/-	2	17	16 (94%)	-	-	-	-	1 (6%)
		?/-	2	6	6 (100%)	-	-	-	-	-
			3	2	2 (100%)	-	-	-	-	-
		?/+	2	18	15 (83%)	-	-	-	-	3 (17%)
			3	4	2 (50%)	-	-	2 (50%)	-	-
		Total		99	81 (82%)			6 (6%)		12 (12%)
Hagino et al. (1999)	IS(A)	+/+	5	8	5 (63%)	2 (25%)	-	-	1 (12%)	-
		+/-	-	-			-	-	-	-
		-/+	5	3	3 (100%)		-	-	-	-
		-/-	5	4	1 (25%)	1 (25%)	-	-	2 (50%)	-
		?/-	-	-			-	-	-	-
		?/+	5	2	2 (100%)		-	-	-	-
		Total	2-4	17	11 (64%)	3 (18%)	-	-	3 (18%)	-

437   [1]GHS = Globally Harmonized System (UN [2003]).

438   [2]Anal = analysis method used to transform the sample data into HET-CAM scores. IS(A) = method described in Luepke (1985); Q = Q-Score,

439   method described in Balls et al. (1995); S = S-Score, method described in Balls et al. (1995).

440   [3]A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category 1); a "-" indicates that the

441   substance was assigned an overall classification of nonsevere irritant (Category 2A or 2B) or nonirritant; a "?" indicates that, due to the lack of

442 appropriate in vivo data (e.g., studies were terminated too early to assess reversibility of effects; insufficient dose volume), a GHS classification
443 could not be made. See Section 6.1 of the Draft HET-CAM BRD for a description of the rules followed to classify the ocular irritancy of test
444 substances tested multiple times in vitro.
445 [4]N indicates number of substances.
446 [5]Number in parentheses indicates percentage of tested chemicals.

447   **Table IV-18. %CV[1] Values for Substances Evaluated Using the IS(B) Analysis Method**
448   **(from CEC [1991])**
449

Calculation	%CV[1] Values
Mean	34.6
Median	33.1
Range	6.6-74.9

450   [1]%CV = percent coefficient of variation.
451

452   **Table IV-19. %CV[1] Values for Substances Evaluated Using the Q-Score Analysis**
453   **Method (from Balls et al. [1995])**
454

Calculation	%CV[1] Values
Mean for All Substances (n=40)	49.83
Median for All Substances	42.50
Range for All Substances	15.09-157.25
Mean for Severe Irritants (GHS[2]) (n=11)	36.26
Median for Severe Irritants	38.93
Range for Severe Irritants	15.35-54.87
Mean for Severe Irritants (EPA[3]) (n=8)	33.54
Median for Severe Irritants	34.81
Range for Severe Irritants	15.35-54.87

455   [1]%CV = percent coefficient of variation.
456   [2]GHS = Globally Harmonized System (UN [2003]).
457   [3]EPA = U.S. Environmental Protection Agency (EPA [1996]).
458

458

459 **Table IV-20. %CV[1] Values for Substances Evaluated Using the S-Score Analysis**
460 **Method (from Balls et al. [1995])**

461

Calculation	%CV[1]
Mean for All Substances (n=5)	84.42
Median for All Substances	71.90
Range for All Substances	68.47-116.4
Mean for Severe Irritants (GHS[2]) (n=2)	81.53
Median for Severe Irritants	81.5
Range for Severe Irritants	68.47-94.59
Mean for Severe Irritants (EPA[3]) (n=2)	81.53
Median for Severe Irritants	81.5
Range for Severe Irritants	68.47-94.59

462   [1]%CV = percent coefficient of variation.
463   [2]GHS = Globally Harmonized System (UN [2003]).
464   [3]EPA = U.S. Environmental Protection Agency (EPA [1996]).

465

466 **Table IV-21. %CV[1] Values for Substances Evaluated Using IS(B) Analysis Method**
467 **(from Spielmann et al. [1996])**

468

Calculation	%CV for IS(B)-10	%CV for IS(B)-100
Mean %CV Value	60.17	35.21
Median %CV Value	42.65	26.22
Range %CVs	0-141.42	0-141.42
Mean %CV Value (Minus Substances Tested in 3 Laboratories)	58.07	34.62
Median %CV Value (Minus Substances Tested in 3 Laboratories)	31.85	21.57
Range %CVs (Minus Substances Tested in 3 Laboratories)	0-141.42	0-141.42

469   [1]CV = coefficient of variation.

470

471

471
472 **Table IV-22. %CV[1] Values for Substances Evaluated Using the IS(A) Analysis Method**
473 **(from Hagino et al. 1999)**
474

Calculation	%CV
Mean for Severe Irritants (GHS[2]) (n=8)	24.4
Median for Severe Irritants	27.0
Range for Severe Irritants	8-39
Mean for Severe Irritants (EPA[3]) (n=6)	23.86
Median for Severe Irritants	26.0
Range for Severe Irritants	8-39

475 [1]%CV = percent coefficient of variation.
476 [2]GHS = Globally Harmonized System (UN [2003]).
477 [3]EPA = U.S. Environmental Protection Agency (EPA [1996]).
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479
480 **3.5   HET-CAM Test Method Historical Positive and Negative Control Data -**
481 **Reanalysis**
482
483 Historical data for positive and negative controls was provided by Johnson and Johnson, Co.,

484 and ZEBET). These data are included in the HET-CAM BRD Addendum (July 2005).